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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).

Docket Number	8642/55	Type a plus sign (+) inside this box	+

INVENTOR(S)/APPLICANT(S)

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TITLE OF INVENTION (280 characters max)

hKIS COMPOSITIONS AND METHODS OF USE

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification	Number of Pages	93	☐ Small Entity Statement
☒ Drawing(s)	Number of Sheets	6	☐ Other (specify)

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☒ A check or money order is enclosed to cover the Provisional filing fees.	PROVISIONAL FILING FEE AMOUNT(S)	150.00
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The invention was made by an agency of the United States Government or under a contract with an Agency of the United States Government.

☒ No.
☐ Yes, the name of the U.S. government agency and the Government contract number are:____.

Respectfully submitted,

SIGNATURE: K. Shannon Mrksich
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Date: 8/21/98
Registration No. 36,675 (if appropriate)

☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

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APPLICANT

GARY J. NABEL, ANN ARBOR, MI; ELIZABETH G. NABEL, ANN ARBOR, MI.

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Foreign Priority claimed 35 USC 119 (a-d) conditions met	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
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HKIS COMPOSITIONS AND METHODS OF USE

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Our Case No. 8642/55

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT

INVENTORS: GARY J. NABEL
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TITLE: hKIS COMPOSITIONS AND METHODS OF USE

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hKIS COMPOSITIONS AND METHODS OF USE

BACKGROUND OF THE INVENTION

It is known that transitions between phases of the cell cycle are catalyzed by a family of cyclin-dependent kinases (Nurs, 1990; Hartwell *et al.*, 1974). In many cells, transit through G1 of the cell cycle and entry into S phase requires a binding and activation of cyclin/cyclin-dependent kinase complexes (CDK), predominantly cyclin D-cdk4,6 and cyclin E-cdk2 (Sherr, 1994; Sherr, 1996).

The cyclin-dependent kinase inhibitors (CKIs) are naturally-occurring gene products which inhibit cyclin-CDK activity and phosphorylation of retinoblastoma protein (Rb), resulting in G1/S growth arrest (D.O. Morgan, 1995; Sherr and Roberts, 1995). CKIs directly implicated in CDK regulation are $p21^{cip1/Waf1}$ (Xiong et al., 1993; Harper et al., 1993), $p27^{Kip1}$ (Pyoshima and Hunter, 1994; Polyak et al., 1994; Coats et al., 1996), and $p16/p15^{INKN}$ (Serrano et al., 1993).

Previous studies of these CKIs were focused on their potential role in malignant transformation. For example, PCT Publication No. WO 95/18824 describes a method for identifying agents capable of modulating the ability of p27 to inhibit the activation of the cyclin E-Cdk2 complex. This PCT publication further provides methods for treating subjects diagnosed with a hyperproliferative disorder, such as cancer and hyperplasia, using these agents. Such agents can be both protein and non-protein moieties.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a novel gene, protein and related biological compositions developed from their ability to bind to the p27 protein. Methods of using the various compositions, for example, in the diagnosis, prognosis and treatment of diseases or conditions associated with cell proliferation (such as cancer, restinosis, artherosclerosis, and angiogenisis) are also provided.

The present invention first provides DNA segments, vectors and the like comprising at least a first isolated gene, DNA segment or coding sequence region

that encodes a hKIS protein, polypeptide, domain, peptide or any fusion protein thereof.

As used herein in the context of the instant compositions, the term hKIS will be understood to include wild-type, polymorphic and mutant hKIS sequences. Wild-type sequences are defined as the first identified sequence, polymorphic sequences are defined as naturally occurring variants of the wild-type sequence that have no effect on the expression or function of the hKIS proteins or domains thereof, and mutant sequences are defined as changes in the wild-type sequence, either naturally occurring or introduced by the hand of man, that have an effect on either the expression and/or the function of the hKIS proteins or domains thereof.

Thus, the invention also includes the provision of DNA segments, vectors, genes and coding sequence regions that encode hKIS proteins, polypeptides, domains, peptides or any fusion protein thereof, where the hKIS protein element comprises at least one mutation in comparison to the wild-type sequence. The mutation may be deliberately introduced by the hand of man, for example, in order to test the function of the changed amino acid, *e.g.*, in p27 binding, RNA binding, kinase activity, and/or other functions. Additionally, the mutation may be a naturally occurring polymorphic change, either isolated from normal cells or introduced by the hand of man.

The hKIS mutation may also be in a purified protein obtained directly from an aberrant cell or may be recombinant protein that has been changed to introduce a mutation that mirrors on identified in a patient. The mutation may result in a truncated hKIS gene or protein, or may result in increased, decreased or undetectable levels of hKIS gene or protein being produced. Where diagnostic or prognostic mutated hKIS genes, proteins and antibodies are concerned, the mutant gene, DNA segment, antibody or even peptide will preferably have specificity for the mutant sequence in preference to the wild-type sequence, allowing effective differentiation between the two, as described in more detail herein below.

The DNA segments and vectors may comprise an isolated gene or coding sequence that encodes a hKIS protein characterized as having the following properties:

Being about 419 amino acids in length;

Comprising an amino-terminal kinase domain, preferrably a kinase domain that phosporylates p27;

Comprising a carboxy-terminal RNA binding domain; and

Binding to p27, as may be assessed by one or more cellular assay systems, such as a yeast or mammalian two-hybrid system that identifies functional proteins associations *in vivo*; or by co-immunoprecipitation of the hKIS and p27 proteins from mammalian cell lysates, or by using one or more *in vitro* assays of protein binding;

It will be understood that while the normal, native, wild-type hKIS protein is defined in terms of these properties and domains, the overall features will generally be the same for hKIS polymorphic and mutant proteins and domains as well. The polymorphic and mutant hKIS genes and proteins can be understood with reference to the wild-type sequences.

The genes and DNA segments of the present invention preferably encode wild-type or polymorphic hKIS proteins, polypeptides, domains, peptides or fusion constructs thereof where the hKIS sequence includes a contiguous amino acid sequence from SEQ ID NO:2, or a biologically functional equivalent thereof. As used herein, the term "contiguous amino acid sequence" will be understood to include a contiguous amino acid sequence of at least about 4, about 6, about 9, about 10, about 12, about 15, about 20 amino acids or so.

The DNA segments and coding regions may encode wild-type, polymorphic or mutant hKIS peptides, *e.g.*, of from about 15 to about 30 or about 50 amino acids in length or so. The hKIS peptides may be lacking in any defined hKIS activity, and may, for example, be used in generating antibodies or in other embodiments. The hKIS peptides or domains may also be deliberately engineered to include a mutation, *e.g.*, in order to prepare antibodies that are specific for a mutated hKIS, particularly where the mutation represents one identified in a patient with a cell proliferation disorder or disease.

type, polymorphic or mutant hKIS protein or peptide free from the environment in which it naturally occurs.

Wild-type, polymorphic or mutant hKIS proteins may be full length proteins, such as being 419 amino acids in length. Wild-type, polymorphic or mutant hKIS proteins, polypeptides and peptides may also be less then full length proteins, such as individual domains, regions or even epitopic peptides. Where less than full length wild-type, polymorphic or mutant hKIS proteins are concerned the most preferred will be those containing predicted immunogenic sites and those containing the functional domains.

Generally, "purified" will refer to a wild-type, polymorphic or mutant hKIS protein or peptide composition that has been subjected to fractionation to remove various non-wild type, polymorphic or mutant hKIS protein or peptide components, and which composition substantially retains its wild-type, polymorphic or mutant hKIS activity, as may be assessed by binding to p27 and forming complexes with p27.

Where the term "substantially purified" is used, this will refer to a composition in which the wild-type, polymorphic or mutant hKIS protein or peptide forms the major component of the composition, such as constituting about 50% of the proteins in the composition or more. In preferred embodiments, a substantially purified protein will constitute more than 60%, 70%, 80%, 90%, 95%, 99% or even more of the proteins in the composition.

A polypeptide or protein that is "purified to homogeneity," as applied to the present invention, means that he polypeptide or protein has a level or purity where the polypeptide or protein is substantially free from other proteins and biological components. For example, a purified polypeptide or protein will often be sufficiently free of other protein components so that degradative sequencing may be performed successfully.

Various methods for quantifying the degree of purification of wild-type, polymorphic or mutant hKIS proteins or peptides will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific p27 binding activity of a faction, or assessing the number of

To determine the region of p27 that is phosphorylated by hKIS, kinase assayf were performed with hKIS and either the carboxy terminal domain of p27 or the amino terminal domain of p27. hKIS was able to phosphorylate the amino (NH_2) but not the carboxy (COOH) domain (FIG. 5). The amino terminal domain of p27 has been shown to encode the domain responsible for inhibition of cyclin/CDK.

p27 and p21, when overexpressed in cells, are capable of preventing the progression of G1 to S (FIG. 6). However, this ability of p27 but not p21 is suppressed when the cells are cotransfected with a vector expressing hKIS (FIG. 6), showing that hKIS is able to specifically counteract the activity of p27 *in vitro*.

Example 2 – hKIS Nucleotide Sequence (SEQ ID NO:1)

ATGGCGGGATCCGGCTGCGCCTGGGGCGCGGAGCCGCCGCGTTTTCTGGAGGCCTTC
GGGCGGCTGTGGCAGGTACAGAGCCGTCTGGGTAGCGGCTCCTCCGCCTCGGTGTAT
CGGGTTCGCTGCTGCGGCAACCCTGGCTCGCCCCCCGGCGCCCTCAAGCAGTTCTTG
CCGCCAGGAACCACCGGGGCTGCGGCCTCTGCCGCCGAGTATGGTTTCCGCAAAGAG
AGGGCGGCGCTGGAACAGTTGCAGGGTCACAGAAACATCGTGACTTTGTATGGAGTG
TTTACAATCCACTTTTCTCCAAATGTGCCATCACGCTGTCTGTTGCTTGAACTCCTG
GATGTCAGTGTTTCGGAATTGCTCTTATATTCCAGTCACCAGGGTTGTTCCATGTGG
ATGATACAGCATTGCGCCCGAGATGTTTTGGAGGCCCTTGCTTTTCTTCATCATGAG
GGCTATGTCCATGCGGACCTCAAACCACGTAACATATTGTGGAGTGCAGAGAATGAA
TGTTTTAAACTCATTGACTTTGGACTTAGCTTCAAAGAAGGCAATCAGGATGTAAAG
TATATTCAGACAGACGGGTATCGGGCTCCAGAAGCAGAATTGCAAAATTGCTTGGCC
CAGGCTGGCCTGCAGAGTGATACAGAATGTACCTCAGCTGTTGATCTGTGGAGCCTA
GGAATCATTTTACTGGAAATGTTCTCAGGAATGAAACTGAAACATACAGTCAGATCT
CAGGAATGGAAGGCAAACAGTTCTGCTATTATTGATCACATATTTGCCAGTAAAGCA
GTGGTGAATGCCGCAATTCCAGCCTATCACCTAAGAGACCTTATCAAAAGCATGCTT
CATGATGATCCAAGCAGAAGAATTCCTGCTGAAATGGCATTGTGCAGCCCATTCTTT
AGCATTCCTTTTGCCCCTCATATTGAAGATCTGGTCATGCTTCCCACTCCAGTGCTA
AGACTGCTGAATGTGCTGGATGATGATTATCTTGGGAATGAAGAGGAATATGAAGAT
GTTGTAGAAGATGTAAAAGAGGAGTGTCAAAAATATGGACCAGTGGTATCTCTACTT
GTTCCAAAGGAAAATCCTGGCAGAGGACAAGTCTTTGTTGAGTATGCAAATGCTGGT
GATTCCAAAGCTGCGCAGAAATTACTGACTGGAAGGATGTTTGATGGGAAGTTTGTT
GTGGCTACATTCTACCCGCTGAGTGCCTACAAGAGGGGATATCTGTATCAAACCTTG
CTTTAA

Example 3 – hKIS Amino Acid Sequence (SEQ ID NO:2)

MAGSGCAWGAEPPRFLEAFGRLWQVQSRLGSGSSASVYRVRCCGNPGSPPGALKQFL
PPGTTGAAASAAEYGFRKERAALEQLQGHRNIVTLYGVFTIHFSPNVPSRCLLLELL
DVSVSELLLYSSHQGCSMWMIQHCARDVLEALAFLHHEGYVHADLKPRNILWSAENE
CFKLIDFGLSFKEGNQDVKYIQTDGYRAPEAELQNCLAQAGLQSDTECTSAVDLWSL
GIILLEMFSGMKLKHTVRSQEWKANSSAIIDHIFASKAVVNAAIPAYHLRDLIKSML
HDDPSRRIPAEMALCSPFFSIPFAPHIEDLVMLPTPVLRLLNVLDDDYLGNEEEYED